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NUTRITION REVIEWS

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NUTRITION REVIEWS

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EARLY EXPERIENCES WITH VITAMIN A—A RETROSPECT

With the exception of the first original thinker every investigator in any science owes a debt to those who preceded him and left for him a record of their thoughts and the observations on which they were based. Any investigator who has contemporaries in his field, provided there is exchange of views and information, owes something to them for suggestions and stimulation. It is my intention in this retrospect to pay respects and acknowledge indebtedness to those experimental inquirers in nutrition whose results I assessed, results which influenced the formulation of the plans of my early experiments which brought to light the existence of the first known fat-soluble vitamin.

While a student at Yale I learned from Chittenden, Mendel, and Underhill much about the writings of Voit, Pfluger, Rubner, Atwater, Benedict, and Chittenden, and about the pioneer researches of Kossel, E. Fisher, Abderhalden, and Osborne on proteins and amino acids in nutrition, and of Pavlov's studies on psychic factors in physiology.

At the Wisconsin Agricultural Experiment Station I became instructor in the department of Professor E. B. Hart, whose studies in the nutrition of cows restricted to single-plant rations, corn, wheat, and oats respectively, and his interest in the nutritive significance of inorganic versus organic forms of phosphorus, were breaking new ground. I was assigned work on his experiments. The teaching of animal nutrition in agricultural colleges at that time (1907) was based largely on the studies of Boussingault, Laws and Gilbert, Rubner, Voit, Zuntz, Armsby, *et al.*, and on the numerous feeding studies which had been made to determine "production values" of different feeding systems for meat, milk, egg, and wool.

The first publication which directed my thinking to experiments with isolated ("purified") food substances was that of E. G. Willcock and F. G. Hopkins (*J. Physiol.* **35,** *88 (1906)*), which described how mice died in fourteen days on a diet of zein (protein), carbohydrate, lard, and a salt mixture, whereas they survived twenty-eight days on the food when a supplement of tryptophan was given. I called these studies to the attention of Dr. S. M. Babcock, emeritus professor of agricultural chemistry at the University of Wisconsin, and suggested that we should undertake with small animals to solve the problem of what constituted, in chemical terms, the simplest diet of an animal. I pointed out the advantages of using small animals whose growth, reproduction and life span would pass quickly in review. He became enthusiastic about the plan, and encouraged me to establish my rat colony, the first in America for nutrition studies.

From a desire to learn more of the history of experimental nutrition studies, during the year 1907 I thumbed the pages of volumes **1–37** (*1871–1907*) of *Maly's Jahresbericht ueber die Fortschritte der Thierchemie,* and acquired much information and a good perspective of the progress of investigations in animal and plant chemistry, the isolation of individual compounds from natural products, the development of analytic methods, digestion, metabolism, and chemical pathology. In the chapters labelled "Gesammptstoffwechsel," I learned about the feeding studies with farm animals, largely patterned after the work of Henneberg and Stohman at the Weende Station in Saxony. I also found and studied and reflected on the papers of J. Forster (*Z. f. Biol.* **9,** *297 (1873)*); G. Bunge (*Ibid.* **9,** *104 (1873)*); N. Lunin ("Inaugural Disserta-

tion, Dorpat" from *Maly's Jahresbericht* **10,** *435* (*1880*)); C. A. Socin (*Z. f. physiol. chem.* **15,** *93* (*1891*)); G. Marcuse (*Pfluger's Arch. f. d. ges. Physiol.* **64,** *223* (*1896*)); W. Falta and C. T. Noegerrath (*Hofmeister's Beitrage* **7,** *313* (*1906*)), and some others, who observed the effects of restricting animals to diets composed of isolated food substances. From these studies I learned the effects of an ash-free diet, of acid-base relationships, of failure of fats, lecithin, cholesterol, sodium-nucleinate, of phosphorus-containing and phosphorus-free proteins, of addition of hematin versus inorganic forms of iron, in preventing prompt nutritive failure of mice, rats, or dogs restricted to diets of isolated food substances.

Lunin (*loc. cit.*), C. A. Pekelharing (*Nederland. tijdschrift v. Geneeskunde* **2,** *111* (*1905*)), and F. G. Hopkins (*The Analyst* **31,** *385* (*1906*)) observed that additions of milk to such diets, or even whey, improved them nutritionally to a remarkable degree, and on the basis of such studies Pekelharing and Hopkins, independently, in 1905 and 1906 respectively, expressed the belief that there existed hitherto unsuspected nutrients in natural foods. Hopkins (*loc. cit*) voiced prophetic views concerning the dietary origin of such diseases as scurvy, rickets and beriberi. Yet even in 1912 no procedure had been developed by means of which the effective study of unidentified nutrients could be made. I was not greatly impressed by Eijkman's great discovery until after the appearance of the important book, "The Vitamins," by Funk, in 1913. From this source I became familiar with the studies of Eijkman, Frazer and Stanton, Grijns, Holst and others, and came to realize the significance for normal nutrition during growth and reproduction of a factor which was involved in the beriberi problem. The studies of Osborne and Mendel solved the difficulty of studying the relative nutritive values of different purified proteins by the inclusion in a diet, believed by them to be the equivalent of a mixture of isolated food substances, of 28.3 per cent of deproteinated whey solids—their "protein-free milk."

In retrospect it is evident that I should have progressed more rapidly beyond sustained efforts to succeed in growing young rats on mixtures of isolated nutrients. Largely through the influence of Pavlov, I was led to persevere in the direction of increasing the number of carbohydrates and fats, and of improving flavor and variety in the food for psychic reasons. The great weakness in my experiments, which were somewhat in advance of others in the same field, lay in the failure to appreciate the extent to which milk sugar might supply unsuspected nutrients, and the failure to comprehend the significance of feces-eating by rats on "purified" diets. Yet the discovery of the first-known fat-soluble vitamin came about while Miss Marguerite Davis and I were observing the effects of such diets. The use of butter fat, egg yolk fat, freshly rendered bacon fat, and other fats, was based on the desire to improve the acceptability of the food, supposed to contain only known substances. Our diets, deficient as they were, were just above the minimum in their content of vitamins and so, with a supplementary supply from coprophagy, the animals were able to distinguish for us the superiority of butter fat and egg yolk fat over lard and olive oil (McCollum and Davis, *J. Biol. Chem.* **15,** *167* (*1913*)).

We next made soap from butter fat, formed an aqueous emulsion of this with the olive oil which had not led to growth response as did butter fat, and then recovered the olive oil from the emulsion and found that it had now acquired the physiologic value of the butter fat. We extended our studies to include vegetable oils from several sources, ether extracts of alfalfa leaf, etc., and thus showed something of the distribution of the new nutrient in nature.

Miss Davis and I had observed with rats,

and Hart and I (E. B. Hart, E. V. McCollum, and J. G. Fuller, *Am. J. Physiol.* **23,** *246 (1909)*) had confirmed with young swine, the fact that corn or other cereal grain requires three kinds of supplements to make it complete nutritionally, *viz.*, protein from another source to supply certain amino acids, a calcium salt and a fat containing the newly discovered fat-soluble vitamin. Miss Davis and I then studied polished rice (McCollum and Davis, *J. Biol. Chem.* **23,** *181, see esp. p. 185 (1915)*) and found that these three additions did not make it capable of supporting growth or well-being. We showed that something was essential which is soluble in water, and that both a fat-soluble and a water-soluble factor were indispensable in the diet of the rat.

The experiments of W. Stepp (*Maly's Jahresbericht* **41,** *492 (1911)*) did not assist our thinking, since he extracted almost all of every vitamin by alcoholic extraction of bread, and found that the addition of even 20 per cent of butter fat did not benefit the animals.

During Farmers Week at the Wisconsin Agricultural College, in 1908, I listened to a discussion following a lecture on swine feeding, in which some farmers maintained that yellow corn is a better hog feed than is white corn. The animal husbandry men were quite positive that there was no difference in nutritive value depending on color. But eleven years later Dr. Steenbock demonstrated that yellowness is associated with vitamin A value. Ten years after this discovery, v. Euler and associates demonstrated vitamin A value in crystalline carotene.

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EXPERIMENTAL POTASSIUM DEPLETION IN MAN

In recent years, marked disturbances in potassium metabolism have been recognized in patients with various types of injury and illness. In particular, individuals with uncontrolled diabetes, prolonged vomiting, intestinal distention, diarrhea, or impaired kidney or adrenal function are prone to demonstrate serious disorders of potassium metabolism (*Nutrition Reviews* **5,** *315 (1947)*; **7,** *3, 296 (1949)*). Muscular weakness, paralyses, cardiac enlargement, high venous pressure, high pulse pressure, and various electrocardiographic changes observed in such patients have been considered to be due to potassium deficiency. D. A. K. Black and M. D. Milne (*Lancet* **I,** *244 (1952)*) point out that when potassium deficiency is present clinically, there is an associated derangement of water and other electrolytes. In an attempt to establish what symptoms and signs might be specifically due to potassium deficiency, they have sought to produce in man potassium depletion not complicated by primary deficits of other electrolytes and water.

Two normal subjects were observed during three to five days of a control period, six to seven days of dietary potassium depletion, and finally three days of repletion. The basic daily intake consisted of 3 l. of milk to which 125 g. of glucose had been added. During the six to seven days of depletion the milk was first passed through a cation exchange resin previously charged with sodium. The procedure reduced the potassium concentration from 43 mEq. per liter to 3 mEq. per liter. At the same time the sodium concentration was increased from 36 mEq. per liter to 111 mEq. per liter. The sodium intake was kept constant by adding the required quantity of sodium (either as chloride or as chloride and bicarbonate in the proportions found in normal extracellular fluid) during the control and repletion periods. The passage through the ion exchange column reduced the calcium concen-

tration from 56 to 27 mEq. per liter and the magnesium concentration from 27 to 8 mEq. per liter. No adjustments for these changes were made. During the repletion period, 10 g. of potassium salt, either as chloride or as a mixture of chloride and bicarbonate, were ingested daily in addition to the milk.

During the period of reduced potassium intake the urinary excretion of potassium fell only gradually from the control levels. This resulted in a significant negative potassium balance, and deficiencies of 268 and 289 mEq. of potassium were accumulated during the week of restriction. Phosphorus balance was similarly negative during this time. The serum potassium levels fell to 3.1 and 3.2 mEq. per liter respectively. Erythrocyte potassium did not fall; no measurements of concentrations in other tissues were made.

Neither subject lost weight despite the fact that the lost potassium corresponded to about 2.5 l. of intracellular fluid. The maintenance of weight appeared to be due to the probable retention of sodium and water as judged by a 10 per cent drop in the venous hematocrits. As mentioned, no direct tissue measurements were made, but from indirect calculations Black and Milne felt that the change in intracellular fluid during the potassium depletion period was one of volume rather than composition.

Alkalosis appeared in both subjects. The partial pressure of carbon dioxide was measured in one subject and found normal. The alkalosis was not predominantly due to a primary renal mechanism since the urine became more alkaline during the depletion period. In one subject, the alkalosis responded temporarily to ammonium chloride, and in both the alkalosis responded to the ingestion of potassium salts, either chloride or chloride and bicarbonate.

During the oral repletion period each subject retained considerably more potassium than had been lost. Black and Milne mention that the amount of potassium retention under similar conditions has been used clinically as a measure of the degree of previously existing potassium depletion. They feel that their observations cast doubt on the validity of such an assumption.

Black and Milne point out that despite the loss of about 275 mEq. of potassium during the depletion period, both subjects carried out their duties as laboratory workers throughout the period of study. No muscular weaknesses, either noted subjectively or as measured by a dynamometer, appeared. The only change in the electrocardiograms was the appearance of U waves. Ballistocardiogram tracings were not altered. There was some loss of capacity for sustained effort and some general apathy which the authors feel may have been due in part to the monotonous diet and the consciousness of self-experimentation.

In view of the paucity of signs and symptoms exhibited by their subjects despite an estimated loss of about 5 per cent of the body potassium, Black and Milne doubt whether potassium deficiency per se is responsible for many of the clinical symptoms and signs commonly ascribed to hypokaliemia. They feel that the state of potassium depletion produced by them is comparable to that described after diabetic ketosis or surgical operations as judged by serum levels and subsequent retention of potassium. However, it is probable that the degree of depletion produced by one week of dietary restriction is not so severe as that existing in those patients exhibiting the muscular weaknesses, paralyses and electrocardiographic changes ascribed to potassium deficiency. These patients often have undergone periods of excessive potassium losses in addition to lowered intakes. The rapid clinical response to potassium therapy is compatible with relating these symptoms to potassium deficiency, although, as pointed out by Black and Milne, there are associated disturbances in water and other electrolytes. In certain animals, dietary restriction of po-

tassium leads to significant cardiac, renal, and muscular changes (*Nutrition Reviews* **1,** *10 (1942)*; **4,** *244 (1946)*; **5,** *216 (1947)*; **8,** *278 (1950)*; **9,** *104 (1951)*) which can be prevented and treated by adequate potassium ingestion. The period of low potassium intake, however, must be prolonged for these signs to appear.

The study of Black and Milne is a step forward in the elucidation of the complicated electrolyte disorders seen clinically. Further studies of this nature are needed.

URIC ACID AND CREATINE METABOLISM IN MAN

The metabolic significance of the nonessential amino acids, in comparison with that of their essential relatives, is frequently disregarded. The more or less specific utilization of glycine in the biosynthesis of purines and creatine has been used to elucidate the functional pathology of these compounds in disease.

J. D. Benedict *et al.* (*Metabolism* **1,** *3 (1952)*) have studied the incorporation of fed N^{15}-labeled glycine into uric acid in man. These studies are based on the demonstration of D. Shemin and D. Rittenberg (*J. Biol. Chem.* **167,** *875 (1947)*) that the nitrogen atom in position 7 of the purine ring is specifically derived from the amino nitrogen of glycine. Benedict and associates reasoned that the rate of appearance of the isotope and the concentration achieved would give information concerning the rate of formation of uric acid. Previous studies with N^{15}-labeled uric acid had shown that in gout the "miscible" pool is increased as compared with nongouty subjects (J. D. Benedict, P. H. Forsham, and DeW. Stetten, *Ibid.* **181,** *183 (1949)*). However, it was not possible to establish the mechanism for this abnormality, and a defect in renal excretion was not satisfactorily eliminated.

Two normal adult males, one patient with gout, and one patient with progressive muscular dystrophy were placed on a creatine-free, low purine and low protein diet. After a control period of five to ten days they were given 100 mg. per kilogram body weight of N^{15}-labeled glycine with breakfast. The isotope concentration of the uric acid from all 4 subjects rose promptly after glycine ingestion and reached a maximum within two to four days. In the normal subjects the decline of the per cent excess of N^{15} atoms was approximately linear from the fourth to the twenty-sixth day. The uric acid from the subject with muscular dystrophy contained a higher concentration of N^{15} than that from the normals. After the sixth day the decline in concentration of N^{15} was linear as in the normal subjects, but the slope was steeper.

The maximum N^{15} enrichment of the uric acid from the gouty subject was three times that observed in the 2 normal subjects. The fall in concentrations was also found to be more abrupt, and only five days were required to reach one-half the maximal concentration, whereas a period of more than twenty-two days was required by the normal subjects. After ten days the decline in isotope enrichment was approximately normal.

A difference between the gouty subject and the normal subjects was apparent in the total conversion of glycine to uric acid during the first ten days. Approximately 0.5 per cent of the ingested glycine nitrogen was excreted as uric acid by the gouty subject as compared with one-third this amount incorporated by the normal subjects.

Interpretation of these results must be provisional because there was a limited number of individuals studied, dictated by the difficult analysis required. The gouty patient's disease was in its relatively early stages, and without renal involvement. His uric acid excretion in the control period averaged 689 mg. per day, which is to be compared with the excretion of 435 and 400 mg. per day by the 2 normal men. It is to be

expected under these circumstances that the rate of conversion of glycine to uric acid would be high.

The authors have speculated concerning the rapid rise and rapid nonlinear fall in isotope concentration which occurred in the gouty patient. From their previous experience with labeled uric acid, referred to above, it seemed certain that the slow linear fall in isotope concentration in the normal subjects was not due to the slow turnover of uric acid itself. The excretion of this substance from the body is prompt. The following factors probably contribute to the prolonged excretion in the present experiments: (1) large size of the pool of uric acid precursors and the slowness of its turnover; (2) delayed incorporation of glycine N^{15} into position 7 of the purine, and of N^{15} from the general nitrogen pool into the other three nitrogen positions. It is suggested that in gout there is a rapid mechanism for the transformation of dietary glycine into uric acid and that an "overproduction" of uric acid is responsible for the large miscible pool.

The conversion of glycine into creatine was measured at the same time that the observations on uric acid were made (M. Roche *et al.*, *Metabolism* **1,** *13* (*1952*)). Creatine appears in the urine of patients with progressive muscular dystrophy in increased amounts. Little direct evidence has been presented as to the most likely of two possible mechanisms. The first mechanism would be the inability of the damaged muscle to retain creatine and the other mechanism would be the decreased ability of the muscle to withdraw creatine from the blood. Muscle creatine is derived both from dietary creatine and the creatine synthesized by the liver and kidney. In the biosynthesis of creatine the amidine group is transferred from arginine to glycine to form guanidoacetic acid; subsequently this latter compound is methylated by methionine to form creatine. Creatine is removed from the blood by muscle, where it exists as such or as its phosphorylated derivative. Evidence suggests that creatinine is formed by the splitting out of phosphoric acid from muscle phosphocreatine (H. Borsook and J. W. Dubnoff, *J. Biol. Chem.* **168,** *493* (*1947*)).

Roche *et al.* (*loc. cit.*) reasoned that if the ability of the muscle to retain creatine were faulty, the creatine in the urine should have the same isotope concentration as muscle creatine. Because creatinine is believed to be formed only from muscle creatine, the isotope concentration of creatinine should reflect the isotope concentration of muscle creatine. If both urinary creatine and creatinine are derived from muscle creatine and creatine phosphate, they should have a similar N^{15} concentration. If the difficulty arose from the inability of the muscle to withdraw creatine from the blood, urinary creatine would represent recently synthesized creatine and not muscle creatine, and should have a much higher isotope concentration than urinary creatinine. The N^{15} analyses of the creatinine excreted proved to be only slightly higher in the dystrophic individual than normal, suggesting that the percentile replacement of muscle creatine was near normal. The isotope concentration of urinary creatine, however, reached a value ten times higher than the level in creatinine in the dystrophic individual after the ingestion of the labeled glycine. The isotope concentration of creatine fell rapidly so that by the eleventh day the isotope concentration of the creatine and creatinine were similar. These data suggested that the diminished functional muscle mass cannot remove all the creatine from the body. The excess creatine is therefore excreted in the urine. Because the total excretion of creatine and creatinine is approximately normal it is unlikely that increased production is operative.

THYROID UPTAKE OF IODINE IN GOITER

The predominant importance of iodine deficiency in the etiology of endemic goiter is conceded by most authors (W. H. Sebrell, *Nutrition Reviews* **8,** *129 (1950)*). This acceptance has been applied widely in the public health measure of fortification of salt with iodine. In communities where endemic goiter was prevalent this program has led to a progressive decrease in incidence of the disease. Radioactive iodine uptake studies have now been reported from several formerly goitrous areas of this country, and the uptakes observed do not differ significantly from nongoitrous areas. It is apparent, therefore, that in this country iodine deficiency no longer exists as a major nutritional problem.

Because the disturbed physiology of the human thyroid in iodine deficiency has not heretofore been defined by the use of modern tracer methods, a joint project was instituted by the Thyroid Clinic of the Massachusetts General Hospital and the Instituto del Bocio de la Universidad Nacional de Cuyo Mendoza, Argentina. A preliminary report of their findings has recently been published by J. B. Stanbury and his collaborators from both institutions (*J. Clin. Endocrinol.* **12,** *191 (1952)*). The countryside about Mendoza is a desert land irrigated for centuries from snow-fed rivers originating in the nearby Andes. Goiter is endemic among the inhabitants of the area and this report concerns the findings in 122 goitrous patients.

The forty-eight-hour thyroid accumulation of I^{131} was measured in 118 of the patients. The percentage uptake by the thyroid found in these goitrous subjects was compared with uptakes by enthyroid subjects in Boston. Although some of the Argentinian subjects had uptakes within the normal range recorded in Boston, there were many who had high uptakes, which in Boston would have been diagnostic of hyperthyroidism.

An attempt was made to correlate the I^{131} uptake with the excretion of I^{127}. The excretion of I^{127} in many of these patients was less than 10 micrograms per day as compared to values of 126, 145, and 293 micrograms per day obtained from the urine of 3 of the Massachusetts subjects. An inverse relation was shown to exist between iodine excretion and the thyroid uptake of I^{131}.

One of the most interesting findings of this project was the slow apparent release of I^{131} from the thyroid gland. The rapid and increased accumulation of I^{131} in the thyroid gland was expected from established principles of thyroid physiology. The incautious observer, however, would have predicted a rapid release of I^{131} from the gland similar to that which occurs in hyperthyroidism. The biologic half life of I^{131} within the thyroid gland was found to be as great as one hundred and fourteen days, a period much longer than normal. In the presence of iodine deficiency, extrarenal losses of iodine were minimal, but the renal clearance of iodide remained unchanged. The true nature of the prolonged half-life of I^{131} in the thyroid gland was elucidated using the blocking agent 1-methyl-2-mercaptoimidazole. Six patients who had been given a tracer dose of I^{131} received this drug. The treatment greatly accelerated the loss of I^{131} from the thyroid. In one case cited, the biologic half-life of thyroid I^{131} fell from fifty-eight days to five and four-tenths days. Concomitantly, there was a twenty-eight-fold increase in I^{127} excretion. The interpretation offered for these findings is that hormone iodine is released by the thyroid at a normal rate, but I^{131} liberated in the tissues by the breakdown of thyroid hormone is rapidly reaccumulated within the thyroid gland. It was possible further to shorten the biologic half-life by the administration of thyrotropin. This suggested that the thyroid glands of these

patients were not subject to maximal endogenous thyrotropic stimulation.

Supplemental iodine was given to four groups of patients with high initial I^{131} uptakes. The iodine dose for each group respectively was 150, 500, 1500, and 150,000 micrograms per day. Despite a supplemental iodine dose of 150 micrograms per day for four weeks, no appreciable fall in accumulating capacity of 4 or 5 patients was measured. A similar tendency existed at higher doses, but the data are incomplete. Because these patients presented no symptoms suggesting the release of excessive amounts of thyroid hormone, the presumption is that thyroid hormone was being stored within the thyroid gland.

A normal response of the anterior pituitary to circulating thyroid hormone was demonstrated by the inhibition of I^{131} uptake by the oral administration of 0.1 g. of desiccated thyroid per day.

Because of the inconsistencies of the older literature the importance of iodine deficiency in the production of endemic goiter has been challenged by I. Greenwald (*J. Clin. Endocrinol.* **6,** *708* (*1946*); *Nutrition Reviews* **6,** *40* (*1948*)). He pointed out the discrepancies between goiter incidence and iodine intake and excretion. One of the additional possible factors in goitrogenesis is the presence of natural goitrogens in food. One such compound has been identified in the edible plants *Brassica* by M. A. Greer, M. G. Ettlinger, and E. B. Astwood (*J. Clin. Endocrinol.* **9,** *1069* (*1949*); *Nutrition Reviews* **8,** *196* (*1950*)). This substance has been shown to be vinyl-thio-oxazolidone. The results obtained by Stanbury *et al.* in Argentina do not suggest that natural goitrogens are important in this area.

The data can be interpreted as showing simple iodine deficiency among those individuals with high radioactive iodine uptakes. The authors attribute the normal and low uptakes of some of their goitrous patients to the sporadic medicinal use of iodine. It is hoped that the full report of this important contribution will not be long delayed.

THE DIETARY TREATMENT OF ATHEROSCLEROSIS IN MAN

The relation between dietary cholesterol, plasma cholesterol, and the occurrence of atherosclerosis in man has been subject to wide interest, extensive discussion, and a few pertinent observations in the last few years (*Nutrition Reviews* **8,** *176*, *341* (*1950*); **9,** *167* (*1951*)). One of the chief difficulties in studies of this kind in man has been the lack of a satisfactory measure of the degree of atherosclerosis in an individual, resulting in the virtual impossibility of determining whether a therapeutic regimen has made a change in the degree of atherosclerosis. Since it is believed that most if not all patients with coronary occlusion and myocardial infarction have coronary atherosclerosis, a group of patients who have suffered this cardiac insult should serve as controls for a similar group for whom a specific therapeutic regimen is designed. Such a study has been undertaken by L. M. Morrison (*Am. Heart J.* **42,** *538* (*1951*)).

In this study 100 consecutive patients with coronary atherosclerosis as determined by previous coronary occlusion and myocardial infarction (established by clinical findings and typical electrocardiographic changes within six months of the study) were observed over a period of three years with regard to recurrent coronary thrombosis, congestive heart failure, mortality rate, serum lipid determinations and body weight. The patients were divided into two groups of 50 each, the division being made by "comparable" alternation. One group of 42 males and 8 females, averaging 60 years of

age, served as controls by being allowed to eat ad libitum an "average American diet" which the author estimates contained between 80 and 160 g. of fat, and 200 to 1800 mg. of cholesterol per day. The treated series comprised 43 males and 7 females whose ages averaged 62 years. This group was given the experimental diet designed to be low in fat and cholesterol. The author states that the fat content was 25 g., and that of cholesterol 50 to 75 mg. daily. The maximal caloric allowance was 1500 calories daily and furnished 320 g. of carbohydrate and 90 g. of protein.

The results in this small group of patients suggest a beneficial effect upon morbidity and mortality in the group given the low-fat, low-cholesterol diet. That is, of the 50 patients allowed to consume the "average American diet," 35 were alive at the end of three years (mortality rate, 30 per cent), whereas, of the 50 patients fed the low-fat, low-cholesterol diet, 43 were alive at the end of four years (a mortality rate of only 14 per cent). Analysis of the deaths revealed that recurrent coronary thrombosis and myocardial infarction occurred seven times in the control group and but three times in the experimental group. Congestive heart failure accounted for death six times in the control group and three times in the low-fat, low-cholesterol fed series.

In the treated series there was a drop in the serum lipids and fractions thereof. The total serum lipid over a three-year period fell from 840 mg. per cent to 571 mg. per cent, neutral fat from 236 to 120 mg. per cent, phospholipid from 292 to 231 mg. per cent, and cholesterol from 312 to 220 mg. per cent.

Clinically, the author noted that many of the patients showed subjective improvement and that there seemed to be a decrease in angina pectoris in the treated group. Weight reduction occurred, as might be expected on a diet as low in calories as that listed. The influence of weight loss per se cannot be described with these data. Medications given were stated by the author to have been administered equally in the two series. They consisted of digitalis, aminophyllin, nitroglycerine and occasional sedatives.

The author states that the figures on a series of patients of this size are not susceptible to statistical analysis. Nevertheless, he feels that the results are significant enough to stimulate further investigation of the influence of a low-fat, low-cholesterol diet in the prevention and treatment of atherosclerosis.

The experiments described were long and arduous. It is unfortunate this or another study cannot be designed and extended to furnish data which *are* susceptible to analysis and critical conclusions.

CORTISONE AND ACTH IN NONTROPICAL SPRUE

Certain cases of steatorrhea which fit the clinical picture of sprue are well known to be resistant to therapy, particularly those classified as nontropical. Full remission of the symptoms and favorable changes in the laboratory findings are difficult to obtain despite the use of vitamin B_{12}, folacin, and liver extract, either alone or in combination and in addition to low-fat, high-protein diets. A number of studies now indicate that temporary remissions in such refractory patients are obtainable with the use of adrenocorticotropic hormone or cortisone.

An early report of the successful use of ACTH was that of T. P. Almy (*Ann. Int. Med.* **34,** *1041 (1951)*), whose patient had had an eleven-year history of diarrhea only temporarily improved by the use of concentrated salt-poor albumin and other measures. During the administration of ACTH there was a rapid, striking improvement in strength and appetite with sub-

sequent reduction in the number of stools and volume of the feces. Weight increase was marked and improvement in the x-ray pattern of the small intestine also was found. Despite these changes, however, hypocalcemia and tetany occurred during the course of therapy when supplements of calcium and vitamin D were discontinued. Hypokaliemic, hypochloremic alkalosis also complicated the patient's course. Appropriate therapy corrected these findings during hormone therapy, but the remission ended shortly after withdrawal of ACTH, and the patient's symptoms returned.

A subsequent study by D. Adlersberg, H. Colcher, and S. R. Drachman (*Gastroenterology* **19,** *674 (1951)*) reports experience with both ACTH and cortisone in 5 patients, all of whom had a majority of the characteristic changes found in sprue at the time of study despite previous therapy. During the period of therapy with these agents the patients received a low-fat, low-sodium, high-protein diet which they had been given previously.

Vitamin A absorption studies were carried out one week before and again during the period of administration of ACTH intravenously in 3 patients, only one of whom, however, was included in the subsequent study of prolonged hormone therapy. Absorption was determined by observing the changes in serum vitamin A over an eight-hour period following the ingestion of 180,000 I. U. of vitamin A in oil. The experimental test was carried out by giving the vitamin one-half hour after the beginning of an eight-hour infusion of 50 mg. of ACTH in 500 cc. of dextrose in water. In these experiments, no demonstrable effect of the hormone on the absorption curve could be found.

More prolonged studies were then carried out using ACTH intramuscularly in doses of 50 to 100 mg. daily. All five courses administered to 4 patients resulted in marked improvement in the sense of well-being and appetite, accompanied by disappearance of diarrhea and the appearance of fewer and formed feces. Vitamin A tolerance curves during this type of regimen revealed improved absorption in 2 patients, no change in one, and in one patient increased fasting vitamin A was found in the serum but the curve during the test remained flat. Hematologic changes could be followed in only the one patient who had had a hematologic relapse prior to treatment. No improvement and no reticulocyte rise took place during a five-day course of ACTH in this subject, but subsequent vitamin B_{12} and folacin therapy yielded a reticulocyte response. Serum albumin levels rose in 2 patients who were so studied, but no other nitrogen or protein investigations were carried out in this series. Subsequent to the withdrawal of ACTH, relapse occurred in all patients within a few days to two weeks.

The effect of cortisone was also observed during eight courses administered to 5 patients. Doses of 100 mg. initially, with subsequent reduction to 50 mg. daily, were used, five courses being given with intramuscular and three with oral therapy. In all courses the results were similar to those seen during ACTH administration. Steatorrhea was decreased, but fecal fat did not become normal, and vitamin A tolerance was found to be improved on one occasion in one patient while all other tolerance curves in this patient and in the remaining patients showed no alteration. Roentgenographic studies in one subject revealed improvement in the small intestine pattern during a prolonged course of cortisone. As in the ACTH studies, however, relapse uniformly took place following cessation of treatment, usually occurring within one to five weeks. Although no details were given, 2 patients were noted to develop hypocalcemia, hypokaliemic alkalosis, and tetany, requiring interruption of hormone therapy.

Also included in these authors' studies is a report of the effect of cortisone in a patient with adrenal insufficiency with

marked gastrointestinal symptoms. Both vitamin A tolerance curves and fasting serum carotene levels improved during cortisone therapy, whereas previous treatment with desoxycorticosterone and salt had failed to change these findings. The authors suggest that this may indicate a specific action of cortisone in enhancing vitamin A absorption.

A more detailed investigation of hormone therapy in nontropical sprue is that of A. B. Taylor, E. E. Wollaeger, M. W. Comfort, and M. H. Power (*Gastroenterology* **20**, *203 (1952)*). Six patients were carefully followed during the administration of cortisone (given orally to 5, intramuscularly to one). Placebo control periods were utilized, and 2 subjects were observed with regard to nitrogen and electrolyte balance and fat excretion. One other patient had fat excretion analyses done, and the remainder had numerous blood chemical determinations. Except for one patient who received vitamin K orally on two days, therapy was confined to diet, a multiple vitamin capsule, and one gram of calcium given orally daily.

The general effects achieved in this series of patients were almost identical with those noted previously with regard to improvement in appetite, strength, and fecal findings. Nocturnal diuresis previously reported by some of these workers (E. E. Wollaeger and B. H. Scribner, *Gastroenterology* **19**, *224 (1951)*), also was lessened but not eliminated. Edema was frequently found during 100-milligram dose periods, but this was diminished or prevented by the use of 50 or 75 mg. doses. The changes occurring during treatment were reversed after cortisone administration was stopped, although the remissions were maintained for varying periods up to approximately thirty-six days.

Steatorrhea decreased in the 3 patients so studied, but in only one did this revert to normal figures. In one patient, increasing the dietary fat from 50 to 100 g. while cortisone was administered at 25 mg. doses markedly increased the fecal fat. Subsequent raising of cortisone dosage to 100 mg. daily reduced the fecal fat. Fecal nitrogen also fell during cortisone administration, and nitrogen balance became slightly more positive than during control periods. The absence of a markedly positive nitrogen balance was explained by a slight increase in urine nitrogen while cortisone was being given. In contrast to the rapid relapse in other features of these patients, withdrawal of the hormone was not followed by increasing fecal nitrogen even after eighteen- and thirty-six-day intervals in the 2 patients studied.

Absorption of vitamin K was increased in all patients as measured by the marked decrease in prothrombin time to normal, usually by the end of six days of therapy. Likewise, serum vitamin A levels rose and in one patient improved absorption occurred during a tolerance test of 7500 I.U. of vitamin A in oil given orally. No significant changes in oral glucose tolerance were observed, however.

Of the other studies carried out, serum albumin concentrations rose, positive balances for sodium and chloride, negative balances for potassium, and inconsistent changes in calcium and phosphorus balance were recorded. As in the previous investigations reviewed (*see above*), hypocalcemia during treatment developed in one patient in this series. No marked alterations in basal metabolic rate were found.

This series of reports indicates that consistently similar improvement in the nutrition of patients with nontropical sprue is obtainable by the use of ACTH or cortisone. The temporary nature of the remissions induced, however, indicate that the fundamental defect in this disease is probably not altered by these hormones. Of some interest in these steatorrhea patients is their apparent ability consistently to absorb steroid substances such as cortisone while only irregularly absorbing vitamin A. These agents appear to offer considerable aid in the

practical handling of acute exacerbations in steatorrhea patients provided the value of low-fat, high-protein diets and of folacin, vitamin B_{12} and crude liver are not overlooked. Supplements of calcium and potassium should also be used during hormone therapy to avoid overt manifestations of deficiency in these electrolytes.

INFLUENCE OF NONPROTEIN CALORIES ON PROTEIN METABOLISM

Modern ideas about the roles of carbohydrate and fat in metabolism have their roots in developments in nutrition which occurred in the nineteenth century. For example, calorimetry of foodstuffs was begun in the 1860's by C. Voit and somewhat later, as a consequence of Voit's work, M. Rubner formulated his "isodynamic law." Earlier in the same century M. Boussingault had reported the results of a "balance study" done on a cow. Among other things, he measured intake and output of nitrogen.

As a result of these developments, the basic tools for the study and elucidation of the relationship between energy and protein metabolism were now available. However, as these technics were widely applied, many of the results appeared contradictory to each other and inconsistent with the isodynamic law, and it is only recently, in the light of further biochemical knowledge, that it has become possible to interpret the findings in a more enlightened fashion.

It is now recognized that many of the difficulties in interpretation resulted from lack of knowledge about (1) the *variability* of protein metabolism, and (2) the *special roles* of the nonprotein foodstuffs over and beyond their roles as sources of energy.

The (more or less) traditional concept of the relationship between nonprotein foodstuffs and protein metabolism was built about the idea of the "nitrogen-sparing" qualities of carbohydrate and fat by virtue of their caloric properties. When adequate energy was not available to the organism from these sources and from body stores, "exogenous" protein and "endogenous" protein were oxidized instead.

A more critical appraisal of nutritional experiments involving study of calorie-nitrogen relationships has made it clear that, although the traditional view was approximately correct, many variables influence this relationship. Among the more important of these are (1) length of experiment, (2) pre-existing nutritional state of the experimental animal or human subject, particularly with respect to stores of protein and fat, (3) specific nature of the source of the protein and of the nonprotein calories. An excellent discussion of these variables is to be found in a recent article by J. B. Allison (*Fed. Proc.* **10,** *676 (1951)*).

In considering the problem of interpretation of nitrogen balance data he takes up the question of protein stores and their influence on the nitrogen balance. One of the examples presented by him is an experiment in which a dog was fed a protein-free diet alternately with a diet containing an amount of nitrogen insufficient to support nitrogen equilibrium in the presence of adequate stores of protein. No restriction was placed on energy intake. As the stores progressively became depleted this same quantity of dietary nitrogen successively produced nitrogen equilibrium and then a positive nitrogen balance. By this, and several other well conceived experiments, Allison makes it clear that nitrogen balances vary with time and tend to drift toward nitrogen equilibrium until stores are depleted or repleted, depending on the calorie and nitrogen intakes used.

He describes the "nitrogen balance index of dietary protein" as a measure with respect to protein metabolism which is relatively constant and, until they are markedly depleted, independent of the

magnitude of protein stores. Briefly, this index is obtained when absorbed nitrogen is plotted against nitrogen balance at various levels of nitrogen intake.

Allison also mentions the part played by specific amino acids in influencing nitrogen balance. For example, when extra methionine is given to dogs on minimal intakes of protein, less body nitrogen is excreted. It is postulated that dietary and body nitrogen meet in a common pool and when the methionine in the diet is not enough to meet the dog's special need for this amino acid, body protein is "raided" for the necessary methionine with waste of many of the other associated protein constituents. The extra dietary methionine meets this special metabolic need and, as a result, protein stores are preserved to a proportionate degree. Thus the limitations of the nitrogen balance as an index of protein metabolism have been well defined by Allison.

Rubner's isodynamic law also has certain increasingly well defined limitations. J. P. Peters has recently appraised the interrelationship of foodstuffs in metabolism (*Yale J. Biol. Med.* **24,** *48* (*1951*)). In his article he makes a distinction between "operative" metabolism and "energy" metabolism. When a foodstuff plays a role in operative metabolism, this implies that it becomes part of the "machinery" of metabolism, not its fuel. Carbohydrate, for example, is more than just a source of energy. As precursor of pyruvate and hence oxalacetate it plays an essential part in the "operation" of the Krebs cycle. In its absence, excessive gluconeogenesis necessarily takes place. Ketosis also ultimately ensues.

Failure to appreciate this dual role of carbohydrate has resulted in some rather extraordinary statements about the relative protein-sparing capabilities of carbohydrate and fat. For example, in M. Sahyun's book, "Proteins and Amino Acids in Nutrition" (*Reinhold Publishing Corp., New York* (*1948*)), an experiment is referred to as evidence for the fact that fat does not spare protein. The experiment referred to is in a standard textbook, and here one finds that the experiment was on only one subject and involved the equicaloric substitution, for three days, of fat for all the carbohydrate in the diet. A negative nitrogen balance promptly replaced nitrogen equilibrium and reached its maximum on the third day of fat administration.

This experiment is subject to criticism on more than one ground, but mainly it exemplifies lack of appreciation of the fact that fat cannot be expected to substitute for carbohydrate operatively as well as calorically. When all the carbohydrate of the diet is removed, the body needs must supply its own from tissue protein. The misunderstanding has been carried to the clinical level by G. B. Ramasarma (*Surg. Gynec. Obstet.* **93,** *105* (*1951*)) who quotes Sahyun's book as authority for the contention that fat does not spare protein. This sequence of references affords an interesting example of how "hearsay" evidence poorly interpreted can be perpetuated in scientific literature.

Actually, if essential quantities of carbohydrate are given, fat does spare protein, by virtue of its caloric properties. H. N. Munro, in a recent review of the complex subject of nonprotein calories and their influence on protein metabolism (*Physiol. Rev.* **31,** *449* (*1951*)) refers to many experiments in which extra calories from fat and carbohydrate spared protein in a comparable manner. T. B. Van Itallie *et al.* (*Metabolism* **1,** *80* (*1952*)) have recently demonstrated the protein-sparing qualities of an oral fat emulsion used as a caloric supplement in the nutrition of human subjects.

Fat, too, may influence protein metabolism in a manner not directly related to its role as a fuel. P. P. Swanson (*Fed. Proc.* **10,** *660* (*1951*)) has shown that when protein-depleted rats are kept at a caloric intake of approximately 25 per cent of requirements, the content of fat in the diet affects nitrogen metabolism. For example,

if the fat content (in calories) is raised from 10 to 20 per cent of the diet under these circumstances, a significant decrease in nitrogen excretion occurs.

The mechanism which underlies this protein-sparing property of fat has not been clarified. However, it appears that extra methionine in the diet may exert a somewhat similar influence on the nitrogen metabolism of such animals.

It is apparent from the well controlled and critical work of Allison and of Swanson that study of the complex influence of calories upon protein metabolism still has much to offer the investigator of nutritional problems.

METABOLISM OF DIETARY CHOLESTEROL BY THE LIVER

When cholesterol is fed in relatively large quantities, blood concentrations of cholesterol increase. Tolerance to dietary cholesterol is decreased in experimental animals on diets which are deficient in protein (T.-W. Li and S. Freeman, *Am. J. Physiol.* **145,** *646, 660 (1946)*). Whether this intolerance is the result of protein deficiency per se or of the hepatic damage induced by it has not been ascertained.

R. O. Bailey and S. Freeman (*J. Lab. Clin. Med.* **39,** *184 (1952)*) have investigated the effects of cholesterol feeding on dogs with liver function compromised experimentally by construction of Eck fistulas. The Eck fistula removes the liver from the portal venous system by a rerouting of blood of the portal vein through the vena cava. In these animals hepatic function is impaired as demonstrated by a decrease in prothrombin content of the blood, reduction in clearance of the dye rose bengal, and an increase in serum alkaline phosphatase.

The diet provided the dogs in these experiments consisted of bread, milk, corn syrup and yeast in proportions providing approximately 178 g. of carbohydrate, 24 g. of fat, and 42 g. of protein daily. Supplements of cholesterol were given in corn oil through a stomach tube. The daily supplement was 0.3 g. cholesterol in 3 ml. corn oil per kilogram of body weight. In control experiments animals received the supplement of corn oil without added cholesterol.

Plasma lipids, including analyses for total cholesterol, free cholesterol, lipid phosphorus, total fatty acids and neutral fat, were determined on fasting specimens of blood obtained at intervals of two weeks from each animal. Analyses for the lipid components of liver were made at the end of each experiment when the dogs were killed.

The feeding of cholesterol in oil in the quantities stated above to normal dogs failed to induce statistically significant changes in plasma levels of total cholesterol, free cholesterol, phospholipid, and neutral fat. Similarly, corn oil alone as a supplement was without effect.

The various lipid constituents of serum in dogs with Eck fistulas fed the basic diet were reduced to values approximately one-half those of normal animals on a similar dietary regimen. Supplements of cholesterol in oil produced an increase in these levels. The mean concentration of total cholesterol in serum of Eck fistula dogs fed the basic diet was 90 mg. per cent. This compared with a value of 386 mg. per cent in these animals after provision of the cholesterol supplement for twenty-four weeks. The mean serum cholesterol level of Eck fistula dogs given corn oil as a dietary supplement for twenty-four weeks was 115 mg. per cent. The ratio of free to total cholesterol remained within the normal range in these dogs when they were fed the basic diet or the basic diet plus corn oil. However, this ratio was increased to 0.40 in Eck fistula dogs fed cholesterol. Serum phospholipid

and neutral fat were also increased during cholesterol feeding, but the degree of increase was not so great as that of cholesterol. The marked increase in serum cholesterol occurring with a relatively smaller rise in lipid phosphorus accounted for a substantial increase in the cholesterol/lipid phosphorus ratio. This ratio was 11.5 in Eck fistula dogs on the control diet and increased to 25.0 at the end of the twenty-fourth week of cholesterol feeding.

Fatty livers were induced in all animals with Eck fistulas fed cholesterol in oil. There was only a slight increase in the lipid content of the livers of normal dogs given the cholesterol supplement. The increased lipid in the fatty livers of the Eck fistula dogs was made up largely of neutral fat and cholesterol esters. Neutral fat rose from an average concentration of 6.4 g. per 100 g. of liver, wet weight, in animals with the fistula and fed the basic diet, to 18.0 g. per 100 g. in other dogs given the supplement of cholesterol. There was a fifty-fold increase in the concentration of cholesterol esters—from 0.22 g. per 100 g. to 10.9 g. per 100 g. when similarly compared. An examination of microscopic sections of these livers revealed the presence of much fat and in certain livers there was evidence of diffuse scarring or fibrosis.

The cholesterol in oil supplements apparently had an unfavorable effect on liver function, since there was an additional decrease in clearance of rose bengal and a further increase in alkaline phosphatase activity of serum of Eck fistula dogs fed the supplement. There were similar changes in liver function, but of smaller degree in normal dogs given cholesterol.

An interesting incidental observation was that 4 of 7 Eck fistula dogs which received cholesterol had peptic ulcers while none of a group of 5 control animals with Eck fistulas fed the basic diet without supplements had this pathologic finding.

The increase in serum cholesterol observed with cholesterol feeding of Eck fistula dogs cannot be explained on the basis of the retention of bile since icterus and hyperbilirubinemia did not occur in these animals. Rather, it would seem that the defects in cholesterol and lipid metabolism were the result of decreased capacity to dispose of ingested cholesterol in animals with impaired liver function. That cholesterol is degraded in the intact organism has been demonstrated by R. G. Gould (*Circulation* **2,** *467 (1950)*), who found that isotopically labelled cholesterol fed to rats and mice led to recovery of C^{14} in the carbon dioxide of the expired air. The observations of Bailey and Freeman (*loc. cit.*) might be interpreted as indicating that the liver is an organ of primary importance in the catabolism of cholesterol. The inability of the damaged liver to degrade effectively ingested cholesterol would account for the accumulation of cholesterol in serum and of cholesterol and neutral fat in the liver.

VITAMIN B_{12}, ANTIBIOTICS AND EGG HATCHABILITY

Several investigators have reported that vitamin B_{12} is essential for hatchability of hen's eggs and that the crystalline vitamin serves as a replacement for animal proteins in the ration of the hen (*Nutrition Reviews* **8,** *303 (1950)*). Some evidence has accumulated to indicate that the prolonged use of all-vegetable rations for laying hens eventually results in a decline in egg hatchability that cannot be corrected with crystalline vitamin B_{12}. Animal feeds, such as dried whey or liver fractions, were necessary to restore good hatchability (*Ibid.* **9,** *181 (1951)*).

The possible role of antibiotics in egg hatchability was recently studied by workers at the University of Idaho (C. F. Petersen, A. C. Wiese, R. V. Dahlstrom, and C. E.

Lampman, *Poultry Sci.* **31,** *129 (1952)*). Ninety-six White Leghorn hens, 5 months of age, were kept in individual wire-bottom laying cages and fed a vitamin B_{12}-low ration of the following composition: wheat bran, 15; oats, 15; barley, 15; corn, 15; wheat, 7; wheat shorts, 7; dehydrated alfalfa meal, 3; soybean oil meal, 15; riboflavin supplement (500 micrograms per gram), 0.6; oyster flour, 1; bone meal, 2.5; salt, 0.5; vitamin A and D oil, 0.4; and manganese sulfate, 0.025 parts. After four months the egg hatchabilities had declined to approximately 20 per cent. The hens were then divided into 6 lots of 16 hens each. Lots 1 through 5 received vitamin B_{12} injections of 0, 0.5, 1.0, 2.0 and 4.0 micrograms respectively per hen per week. Lot 6 received a dietary supplement of 3 per cent of fish meal. After eight weeks on these treatments the hatchabilities for lots 1 through 6 were 11, 58, 85, 87, 94 and 88 per cent respectively Weekly injections of 1 microgram of vitamin B_{12} per hen appeared to support normal egg hatchability.

The vitamin B_{12} injection and fish meal supplement were then discontinued and the rate of decline in egg hatchability was determined. After an eight-week period the hatchabilities for lots 1 through 6 were 40, 18, 4, 20, 55 and 91 per cent respectively. There was fairly good correlation between the level of vitamin B_{12} supplied during the previous supplemental period and the subsequent decline in egg hatchability. The lot of hens previously supplemented with fish meal (lot 6) maintained a high level of egg hatchability throughout the period, suggestive that this substance may contain some factor or factors essential for hatchability in addition to vitamin B_{12}.

Following twelve weeks of feeding of the basal diet, hatchability had dropped to 22 per cent or less in lots 1 through 5 and was 68 per cent in lot 6. At this time various combinations of antibiotics and vitamin B_{12} were tried. Three micrograms of vitamin B_{12} per kilogram of ration increased hatchability to 75 per cent after eight weeks. Streptomycin or aureomycin alone (50 mg. per kilogram of ration) did not improve hatchability. Low levels of vitamin B_{12} alone (0.75 micrograms per kilogram of diet) were as effective as low levels of this vitamin combined with the antibiotics.

In a second experiment (C. F. Petersen, C. E. Lampman, and A. C. Wiese, *Poultry Sci.* **31,** *132 (1952)*) these workers investigated the influence of fish meal and an animal protein factor supplement (obtained from aureomycin production) on hatchability and quality of chicks hatched.

An all-vegetable protein ration decreased hatchability to near zero in six months when the hens were kept on wire floors. When the same diet was fed to hens that were kept on built-up litter good hatchability was maintained. Good hatchability was likewise maintained with dietary supplements of 1 or 5 per cent fish meal. A supplement of 0.25 per cent of animal protein factor did not support good hatchability unless the hens had access to built-up litter.

Of interest in this study was the marked difference in the growth that was obtained when the hatched chicks were fed a vitamin B_{12}-deficient diet and the same diet supplemented with vitamin B_{12}. In all cases vitamin B_{12} stimulated chick growth. This stimulation was most marked in the chicks from hens reared on wire and receiving animal protein factor supplement. Even with access to litter and a 1 per cent fish meal supplement, the hens did not transfer enough vitamin B_{12} to the egg to permit normal growth of the hatched chicks. A level of 5 per cent fish meal and animal protein factor supplement produced chicks with sufficient carryover of vitamin B_{12} to permit good growth for four weeks on a vitamin B_{12}-low diet.

In view of the accumulating evidence that still unidentified factors are of importance in egg hatchability, these results are not surprising. It is possible that the fish meal and the access to built-up litter supplied such unknown factors.

H. Yacowitz, R. F. Miller, L. C. Norris,

and G. F. Heuser (*Poultry Sci.* **31,** *89 (1952)*) also investigated the vitamin B_{12} requirement for egg hatchability, and the level of vitamin B_{12} in the egg as influenced by diet. These workers noted that an all-vegetable diet containing 25 per cent of protein resulted in more rapid depletion of vitamin B_{12} reserves of hens than a similar diet containing 16 per cent of protein. On the former diet the vitamin B_{12} requirement for normal hatchability appeared to be not greater than 0.2 micrograms per 100 g. of diet. Eggs from the same hen showed marked variability in their vitamin B_{12} content. Vitamin B_{12} appeared in greatest concentration in the yolk, and the yolk content was correlated with level of hatchability. When hatchability had declined to approximately 30 per cent, the vitamin B_{12} content of the egg yolk was 0.6 millimicrograms per gram. Hatchabilities of 80 per cent or higher yielded eggs containing approximately 1.8 millimicrograms of vitamin B_{12} per gram of yolk. It was concluded that the vitamin B_{12} requirement of the embryo for normal hatchability would be approximately 2.5 millimicrograms per gram of yolk.

Quantitative data is thus gradually accumulating concerning the requirements of poultry for some of the newer nutritional factors. It must be remembered, however, that most poultry nutritionists are accumulating a vast store of information and attempting to derive quantitative data on practical rations. From a practical point of view this is perhaps desirable, but from the scientific point of view data obtained with purified diets would seem to deserve greater consideration.

At present it is difficult and usually impossible to attempt to evaluate the work of the various groups of investigators in this field because the nature of the all-vegetable ration in common use varies tremendously. Presumably, plant materials contain traces of the nutrients under study, and furthermore their concentration in different plant species probably varies.

COBALT DEFICIENCY IN LAMBS

The discovery of cobalt as an essential nutritional element dates back to 1935 when Australian workers noted a wasting disease in cattle and sheep that could be cured with inorganic salts of cobalt. The quantitative needs for cobalt have not been definitely established. Available evidence indicates that when the dry matter of forages contains less than 0.1 mg. per kilogram of cobalt a deficiency may result. Doses of 0.1 mg. of cobalt daily to cattle or sheep usually result in prompt recovery from cobalt deficiency.

J. Stewart of Edinburgh, Scotland, has recently induced cobalt deficiency experimentally (*Brit. J. Nutrition* **5,** *320 (1951)*). This work was initiated primarily to prove that the disease condition commonly referred to as "pining" or "marasmus" is indeed a cobalt deficiency, and to demonstrate that cobalt per se is the preventive agent.

A diet was devised that was made up of foodstuffs sufficiently remote from pasture material to avoid the criticism that some unknown pasture factor other than cobalt might be involved in "marasmus." The daily ration contained 1100 g. of flaked corn, 250 g. of hay, and 60 g. of a mineral mixture of ground limestone, steamed bone meal and crude rock salt. The hay, grown on cobalt-deficient soil, was fed at the minimum level necessary to insure normal rumen function. The daily ration contained 0.0687 mg. of cobalt, based on spectrographic assay.

The diet was fed to pregnant ewes which were housed in concrete bins. Certain of the ewes received an additional supplement of

10 mg. of cobalt weekly as cobalt chloride. All lambs were weaned ten weeks after birth. Those from control ewes were given supplements of 10 mg. weekly of cobalt throughout the experiment. Lambs from ewes not receiving the cobalt supplement were fed the cobalt-low diet alone after weaning. The ration listed above was originally fed at low levels and was gradually increased so that by 16 weeks of age the lambs were receiving the full ration. Ten control and 10 cobalt-deficient lambs were reared under this dietary treatment.

Lambs receiving the cobalt-supplemented ration made normal weight gains and reached 100 pounds live weight in about forty weeks. Those receiving no cobalt supplement made normal progress until the tenth to the fourteenth weeks when they weighed approximately 40 pounds. After this their body weights either remained steady or decreased. If they were continued on the deficient diet the typical signs of cobalt deficiency developed, namely, extreme emaciation and anemia. Several of the deficient lambs responded immediately when given the supplement of 10 mg. of cobalt weekly.

The ration used by Stewart thus appeared to meet the nutritional requirements of sheep except for cobalt, and should facilitate work on the physiologic action and other aspects of cobalt metabolism.

These areas of research definitely require attention. It was pointed out recently (L. A. Maynard, "Animal Nutrition," *3rd edition, McGraw-Hill Inc., New York (1951)*) that studies thus far conducted have provided little basis for accurately diagnosing a deficiency of cobalt in cattle or sheep.

GROWTH FACTORS FOR POULTRY

Since the isolation of vitamin B_{12} in 1948, poultry nutritionists have devoted much attention to whether or not other unidentified factors may be present in animal products that are essential for chick growth. Some of these efforts have also involved studies with turkey poults.

H. Menge, G. F. Combs, and M. S. Shorb (*Poultry Sci.* **28,** *775 (1949)*) presented evidence that growth-stimulating factors, other than vitamin B_{12}, were present in dried whey and certain liver preparations. C. W. Carlson *et al.* (*Ibid.* **28,** *750 (1949)*) made similar claims for fish meal and dried brewers' yeast. J. E. Savage, B. L. O'Dell, H. L. Kempster, and A. G. Hogan (*Ibid.* **29,** *779 (1950)*) also reported growth stimulation from liver in the presence of an adequate intake of vitamin B_{12}. Combs *et al.* (*J. Biol. Chem.* **182,** *727 (1950)*) reported further studies in which it was revealed that a refined liver paste dialysate contained two growth factors essential for chickens which were not identical with vitamin B_{12}. W. W. Cravens, H. W. Bruins, M. L. Sunde, and E. E. Snell (*Fed. Proc.* **10,** *379 (1951)*) made the observation that two unidentified growth factors, one present in torula yeast and the other in wheat bran, were required for chick growth. G. O. Kohler and W. R. Graham (*Poultry Sci.* **30,** *484 (1951)*) claimed that a factor present in leafy green vegetables stimulated chick growth. The above cited claims, which bear evidence of the need of chicks for still unidentified growth factors, were based mainly on work with purified diets.

The growth responses obtained by the above investigators from these unidentified growth factors are not considered to be related to the growth stimulatory action of antibiotics reported by E. L. R. Stokstad and T. H. Jukes (*Proc. Soc. Exp. Biol. Med.* **73,** *523 (1950)*). A. C. Groschke (*Poultry Sci.* **29,** *760 (1950)*) presented evidence, however, which indicated that certain anti-

biotics exerted a sparing action on the requirement of the chick for the unidentified growth factor in dried whey.

In some recent extensive studies reported by H. Menge, G. F. Combs, P.-T. Hsu, and M. S. Shorb (*Poultry Sci.* **31,** *237* (*1952*)) further evidence was presented which indicated that two distinct unidentified factors are required by the chick for rapid growth. One of these is supplied by a 70 per cent alcohol-insoluble portion of the aqueous extract of liver subjected to enzymatic action, or by a similar 70 per cent alcohol-insoluble fraction of a hot water extract of whole liver, or by dried brewers' yeast. The other is supplied by dried whey. Most of the results were obtained on a purified diet in which the alpha protein of soybeans served as the protein source. The diet was fortified with amino acids and vitamins in adequate amounts to meet requirements for the growing chick.

Chicks from pullets housed on wire and fed a ration devoid of animal proteins responded more to the factor present in liver than progeny of hens housed on litter and fed a complete breeder ration. Progeny of hens housed on litter and fed a complete breeder ration throughout one egg-laying year still responded to the factor present in whey, but not to the factor present in liver or yeast. Under maximum depletion conditions, growth responses of 15 to 20 per cent above basal growth responses were usually obtained when sources of the unidentified factors were supplied. These workers emphasize that the variability in results reported from various laboratories may be largely explained by differences in carry-over of the unidentified factors from hen to egg. The factor present in liver is apparently quite stable to alkali since Menge and associates (*loc. cit.*) noted that treatment with steam at 100°C. for thirty minutes at pH 11.3 did not influence its activity.

Of interest in connection with the importance of the carry-over from hen to chick is the recent report by G. O. Kohler and W. R. Graham (*Poultry Sci.* **31,** *284* (*1952*)). These workers noted a seasonal variation in the requirements of chicks for an unidentified growth factor present in the juice of forage crops, the factor reported initially as essential in rat and guinea pig nutrition (Kohler, C. A. Elvehjem, and E. B. Hart *J. Nutrition* **15,** *445* (*1938*)). Seasonal effects were, interestingly enough, also noted in the earlier work with rats and guinea pigs. In the recent report by Kohler and Graham it was noted that chicks obtained during the months when good range grass was available to the hens had a much lower requirement for the grass juice factor than chicks obtained when good range was not available. In fact, the need for the factor often could not be demonstrated at all when progeny were used that had a history of good grass range in the maternal diet.

More and more evidence is thus accumulating that there are still unidentified nutritional factors that are important in poultry nutrition. It would seem that if any uniformity in results is to be expected by the several groups of workers interested in this problem, it will be important to select progeny of known nutritional background. The importance of maternal nutrition as a determining factor in the nutrition of offspring is perhaps more strongly indicated in the field of poultry nutrition than in any other area at present. Another factor of importance is the conditioning induced by metabolic stimulants. In the recent work by Menge and associates protamone, an iodinated product of casein that stimulated the basal metabolic rate, was routinely included in the experimental diets.

DIETARY FACTORS IN DAIRY CATTLE NUTRITION

For the past decade C. F. Huffman and his associates at the Michigan Agricultural Experiment Station have been engaged in dairy cattle nutrition studies. These workers noted that when dairy cows were fed a ration of alfalfa hay alone they usually made efficient use of the total digestible nutrients in such a ration for two to three months; however, after this time milk production usually declined rapidly. This decline in milk production was concluded to be the result of the gradual depletion of some unidentified nutritional factor or factors. When part of the hay was replaced by an equal amount of total digestible nutrients in the form of concentrates, good milk production was restored, an indication that the missing dietary factors were present in such concentrates as corn and wheat. Corn starch or corn sugar, however, did not contain the factor or factors (Huffman and C. W. Duncan, *J. Dairy Sci.* **25,** *507 (1942)*; **27,** *821 (1944)*).

The concept that concentrates contained dietary essentials for dairy cows has been questioned (L. A. Moore, *J. Animal Sci.* **9,** *663 (1950)*). The increase in milk production resulting from the replacement of part of the total digestible nutrients in hay with equivalent total digestible nutrients in concentrates decreased the total fiber intake, and probably increased the net energy value of the better balanced feed mixture. This criticism, however, seems to overlook the fact that corn sugar and corn starch did not contain these dietary essentials as reported by the Michigan workers.

Recently C. F. Huffman and C. W. Duncan (*J. Dairy Sci.* **35,** *30 (1952)*) have investigated the effects of a high fiber feed (peanut hulls) combined with concentrate (corn) on milk production in dairy cattle previously depleted of the lactation factors by the feeding of hay alone.

Eight milking Holstein cows which had been depleted on a ration of hay alone were used in the study. Four of the cows were continued on hay alone and served as controls. The remaining 4 cows were used to determine the coefficients of digestibility of the hay ration alone and again after 15 pounds of the hay had been replaced with 6 pounds of corn and 9 pounds of peanut hulls. The effect of the switch in ration on milk yield was also determined. The hay ration alone averaged 51.0 per cent in total digestible nutrients compared with a value of 48.9 for the hay-corn-peanut hull ration.

When the cows were switched from the all-hay ration to the hay-corn-peanut hull ration, milk production (corrected to 4 per cent fat milk) increased from an average of 69 pounds to an average of 76 pounds per 100 pounds of total digestible nutrients consumed. During this same period the control cows on the all-hay ration declined in milk production at a rate of approximately 7 pounds per 100 pounds of total digestible nutrients consumed.

The results of this study illustrate some of the imperfections commonly in use for determining the nutritional value of various feeds for dairy cows. For example, the hay-corn-peanut hull ration which stimulated milk production contained considerably less calculated net energy and starch equivalent values and more crude fiber than the all-hay ration. The average hay ration contained 39.3 therms per cent (39.3 therms per 100 pounds or 0.87 calories per gram) of net energy compared with 23.7 therms per cent (23.7 therms per 100 pounds or 0.52 calories per gram) for the hay-corn-peanut hull ration. The latter contained 28.8 per cent starch equivalent compared with 32.9 per cent for the former. The hay-corn-peanut hull ration contained 38.0 per cent crude fiber compared with 34.6 per cent in the all-hay ration.

The results support the modern concept that balance in a dairy ration is highly important and reaffirm the earlier views of

the Michigan investigations that concentrates supply some unidentified factor or factors needed for efficient milk production.

In another recent study (C. F. Huffman, C. W. Duncan, and C. M. Chance, *J. Dairy Sci.* **35,** *41* (*1952*)) 17 Holstein cows were used in twenty-five trials to determine the effect on milk production when part of the hay was replaced with grain plus either wheat straw, oakwood meal or peanut hulls. The cows had been depleted of the unidentified lactation factors by feeding hay alone.

Wheat straw, fed with grain to replace part of the hay in the all-hay ration, stimulated milk production. Feeding oakwood meal as a source of indigestible carbohydrate along with grain and hay also increased milk production. However, milk production was stimulated to a greater extent with grain alone, indicating that the oakwood meal had some depressing effect. The trials with peanut hulls combined with grain and hay confirmed the earlier results in that increased milk production was consistently observed when this ration followed the feeding of an all-hay ration. The increase in milk flow again could not be explained on the basis of calculated net energy intake even when a zero value was used for the peanut hulls in the calculations, since the milk production increased in all trials where calculated net energy values were the same as or less than those of the all-hay ration.

The bacteria in the rumen have always been considered to be of major importance in the nutrition of cattle. Whether or not the unidentified factor or factors proposed by the Michigan workers exert their action through the rumen microorganisms has not yet been determined.

Of more important practical significance, perhaps, is the extent to which different hay crops may be deficient in the lactation factor or factors. The authors suggest that one of the major objectives of forage crop research should be to produce hays high in lactation factors to reduce the amount of grain-feeding.

BODY COMPOSITION

The composition of the human body represents an important facet of the nutritional status (nutriture). The two principal approaches applicable to the living man are based on determination of (1) the specific gravity of the body, first used for dogs by W. Kohlrausch (*Arbeitsphysiol.* **2,** *23*, *46* (*1929/1930*)) and applied to man by A. R. Behnke (*Harvey Lectures* **37,** *198* (*1941/42*)), and (2) the total body water estimated by the degree of dilution of a substance in the extracellular and intracellular fluid. The use of antipyrine (R. Soberman *et al.*, *J. Biol. Chem.* **179,** *31* (*1949*)) and of urea (R. A. McCance and E. M. Widdowson, *Proc. Roy. Soc.* (*London*) *B* **138,** *115* (*1951*)) substantially reduced the heavy demands on time and requirements for special equipment involved in the use of tritium oxide (N. Pace, L. Kline, H. K. Schachman, and M. Harfenist, *J. Biol. Chem.* **168,** *459* (*1947*)), and made the method applicable to laboratory and clinical research.

Using the dilution of antipyrine as a measure of body water, J. M. Steele, E. Y. Berger, M. F. Dunning, and B. B. Brodie (*Am. J. Physiol.* **162,** *313* (*1950*)) measured the water content of sizable groups of normal individuals. In 51 men the mean value was 32.7 liters, standard deviation 5.5; in 31 women, 26.4 ± 4.5 liters. In reference to body weight, the means correspond to 52.7 and 44.6 per cent. It is unfortunate that the height of the individuals was not given so that a rough estimate of their degree of overweight could be made. Using the formula

$$\text{per cent fat} = 100 - (\text{per cent water}/0.732)$$

given by E. N. Rathbun and N. Pace (*J. Biol. Chem.* **158,** *667* (*1945*)) we obtain mean values of 28 and 39 per cent of "excess" fat. These are high but not excessive values, in view of the age of the subjects (51 and 45 years, respectively).

The factor of age, and the related factor of fatness, must be kept in mind in comparing the data of Steele *et al.* (*loc. cit.*) with those reported by E. F. Osserman, G. C. Pitts, W. C. Welham, and A. R. Behnke (*J. Applied Physiol.* **2,** *633* (*1950*)). Their mean percentage of water from antipyrine was 61.1 per cent—a value substantially higher than that reported by Steele *et al.* This simply reflects the fact that the group was considerably leaner (16.5 per cent fat using 73.2 as the percentage of water in lean body mass; 15.0 per cent fat assuming 71.8 as the water content of the "fat-free" body). Osserman *et al.* also determined on their sample of 81 men the specific gravity. The fat content estimated from specific gravity and from total body water agreed very closely, both with regard to range and to the mean values. Spearman's coefficient for the rank order correlation for fatness estimates based on the two methods was high (0.9). These data supplement in an important way the earlier study by W. J. Messinger and J. M. Steele (*Proc. Soc. Exp. Biol. Med.* **70,** *316* (*1949*)) which indicated that the values of fat calculated from specific gravity and total body water determined by antipyrine agreed fairly well.

Steele *et al.* (*loc. cit.*) reported three sets of data important for the evaluation of the antipyrine method as a measure of total body water. The reproducibility of body water measurements appears fairly satisfactory, although four successive determinations on 3 individuals, made two to three days apart, do not provide an adequate basis for judgment in this matter. Actually, the range of values obtained on the same individual was not inconsiderable (3.6, 4.0, and 4.9 liters). The magnitude of the "error of measurement" of total body water from antipyrine demands further investigation.

Two sets of data deal with the validity of method, as differentiated from its reliability (repeatability). The volume of body water, measured in a group of 9 edematous individuals, was considerably higher than in the "normal" group. It should be noted that referring the water content to the actual body weight is not a satisfactory procedure. The authors commented that 5 out of 9 markedly edematous patients fell within the "normal" range of body water. Because fat carries with it much less water (6 to 20 per cent) than other tissues, it is preferable to relate the volume of body water to the estimated lean body mass. Otherwise, as Steele *et al.* (*loc. cit.*) commented, an obese person may have a relatively small proportion of body water even at times when edema is clinically present.

An observation important for the validation of the method consisted in making total body water measurements before and after the removal of ascitic fluid in 2 patients. The weight decrements of 7.0 and 5.8 kg., respectively, were closely paralleled by the changes in the antipyrine space (decreases of 6.8 and 6.2 liters).

Valuable information on the nutritional status is provided by determining other fluid spaces. Plasma volume and thiocyanate space were studied in famine edema and during recovery (A. Henschel, O. Mickelsen, H. L. Taylor, and A. Keys, *Am. J. Physiol.* **150,** *170* (*1947*)). The thiocyanate space, expressed as cubic centimeters of fluid per kilogram of body weight, increased on the average by 45 per cent in semistarvation. Excess thiocyanate space was related to clinical edema, but did not manifest itself clinically until the excess reached about 10 per cent of body weight.

E. Y. Berger and co-workers (*Am. J. Physiol.* **162,** *318* (*1950*)) used the dilution of sodium bromide and inulin as measures of the "extracellular" space, in order to

obtain the intracellular space by subtracting the extracellular fluid from the total body water measured by the dilution of antipyrine. The bromide space (about 28 per cent of body weight in men, 25 per cent in women) proved to be larger, the inulin space (15 per cent of body weight) smaller than the thiocyanate space (about 23 per cent in normal young men). Bromide diffuses relatively rapidly (within two to four hours). Inulin diffuses slowly and is excreted fairly rapidly; a continuous intravenous infusion is necessary to attain equilibrium and a correction must be made for the inulin excreted in the urine in calculating the inulin space.

In addition to such criteria as the rate of distribution in the body fluids and attainment of diffusion equilibrium throughout the body, necessary for measuring the volume of fluid in which the substance is distributed, the *absolute* value of the "extracellular space" is important. In using these methods for analysis of body composition the substance which approximates the "anatomic" extracellular space, *i.e.*, a substance which is completely excluded from the cells, is to be preferred. Inulin appears to fulfill this requirement, but the advantages of simplicity of determination, rapid equilibration and slow renal excretion of sodium thiocyanate account for the fact that the latter substance has been used most widely (M. F. Levitt and M. Gaudino, *Am. J. Med.* **9,** *208* (*1950*)). Perhaps a workable correction could be applied to reduce the thiocyanate space which is increased due to the fact that thiocyanate enters the red cells and some tissues.

Berger *et al.* (*loc. cit.*) broke down the total body weight into total body water, extracellular space (inulin), intracellular space (total water minus inulin space), fat (estimated from total body water and body weight), and "solids." A similar procedure was applied by R. A. McCance and E. M. Widdowson (*loc. cit.*), who obtained the total body water by the urea dilution method. Making an allowance for minerals, body fat was estimated by difference between the total weight and the cell mass plus the extracellular fluid, determined as the thiocyanate space (*Nutrition Reviews* **10,** *14* (*1952*)).

Data on the effect of undernutrition on body fluids were reported by E. M. Widdowson and R. A. McCance ("Studies of Undernutrition, Wuppertal 1946–9," *Chapt. 9, Med. Res. Council, London, Special Report Series* **275** (*1951*)). Four undernourished men had a larger extracellular (26.3 versus 21.9 per cent of body weight) and a smaller intracellular (35.8 versus 39.9 per cent of body weight) fluid volume than 5 normal controls. The latter fact indicates a decrease in the muscular and glandular tissues. Of special interest is the comparison of 10 men studied on their return from Russian war-prisoner camps and after eight weeks of abundant food intake. The body weight increased from 57.5 to 72.9 kg., total water decreased (from 69.3 per cent of body weight to 57.3), as did the extracellular fluid (from 38.6 to 23.9), while the cell water showed a small relative (from 30.7 to 33.4 per cent) and a large absolute increase (+6.8 liters). On the assumption that the intracellular water constitutes 67 per cent of the cell mass, 6.8 liters would correspond to 10.1 kg. of "active tissues," principally muscle. The total weight gain of 15.4 kg. may be then partitioned as follows: gain in "muscle" mass, 10.1 kg.; loss of extracellular fluid, 4.8 kg.; gain in fat, by difference, 10.1 kg.

Valuable data on body composition and, specifically, on estimation of body fat from measurements *in vivo* of body water by use of antipyrine in cattle, were reported by H. F. Kraybill, O. G. Hankins, and H. L. Bitter (*J. Applied Physiol.* **3,** *681* (*1951*)). The research workers in the field of animal husbandry have long been aware of the fact that the chemical composition and gross tissue morphology are greatly affected by breeding and feeding. Much of our funda-

mental knowledge on body composition, its relation to age and to qualitative as well as quantitative differences in the food supply came from the agricultural experiment stations (*cf.* S. Brody, "Bioenergetics and Growth," *Reinhold Publishing Corp., New York* (*1945*)). J. A. Murray (*J. Agr. Sci.* **12,** *103* (*1922*)) pointed out that the chemical composition (moisture, protein, ash) of the fat-free body in farm animals is remarkably constant. The concept of chemical maturity, defined as the age at which the composition of the lean body mass approaches constancy and attained in different mammal species at different times, was proposed and documented quantitatively by C. R. Moulton (*J. Biol. Chem.* **57,** *79* (*1923*)).

In the study by Kraybill *et al.* (*loc. cit.*) body water measurements were made on 30 head of beef cattle consisting of mature steers, heifers and cows varying in age and in calorie nutriture. The body water values, expressed as percentage of body weight, varied from 43.9 to 63.3 per cent, with a mean of 54.0 per cent. The most important contribution of the study is the direct validation of the method for the analysis of body composition. The percentage of separable adipose tissue and of ether extract in the carcass was calculated from a physical and chemical analysis of a portion of the wholesale rib cut (ninth to eleventh rib), with mean values of 25.9 per cent (range 16.0 to 39.5) and 26.4 per cent (range 17.1 to 39.3), respectively. These values refer to 26 crossbreds and Herefords for which both values (physically separable adipose tissue and ether extract) were available. The separable fat in the whole group of 30 animals represented 25.2 per cent of body weight. The value of ether extract calculated from the average water content was 26.2 per cent. In this calculation 73.2 was used as the figure for the water content of the lean body mass.

More important than the good correspondence between the average values of body fat, derived from antipyrine and from a chemical analysis of a representative sample of the carcass, is a close parallelism between these variables in individual cases. The correlation coefficient, calculated by the reviewer from the tabular data, is very high ($r = 0.962$) and demonstrates that the antipyrine technic is useful for ascertaining the composition of the individual animal at different stages of growth and fattening. There was also a high correlation between the fat contents estimated from total body water and from the specific gravity of the eviscerated cattle ($r = 0.986$), providing a valuable cross validation for the latter technic.

The relation of body water content to body build in a group of healthy men was studied by C. W. Dupertuis *et al.* (*J. Applied Physiol.* **4,** *364* (*1951*)). The body build was characterized in terms of three "components" of the somatotype, proposed by W. H. Sheldon, with S. S. Stevens, and W. B. Tucker ("The Varieties of Human Physique," *Harpers, New York* (*1940*)), labeled endomorphy (round softness), mesomorphy (muscular solidity) and ectomorphy (linearity-delicacy); *cf.* G. Draper, Dupertuis, and J. L. Caughey, "Human Constitution in Clinical Medicine," *Hoeber, New York* (*1944*). The ratings are made on a scale extending from 1 to 7. Of interest is a comparison of two groups of men, equal in height (70 inches). The first group was "overweight" (190 pounds), with a low total body water (53.8 per cent), and a high endomorphy rating (4.4). The second group was lighter (156 pounds), had a high water content (67 per cent), and a low mean endomorphy rating (2.5). The correlation coefficient between endomorphy ratings and percentage of body water was fairly high ($r = -0.797$) and an equation was set up for predicting the water content from somatotype ratings. In another paper a high positive correlation between endomorphy ratings and percentage of body fat, estimated from specific gravity, was reported (*J. Applied Physiol.* **3,** *676* (*1951*)).

These data indicate the possibilities inherent in the use of photographs for the purposes of systematic evaluation of nutriture. An appraisal system, specifically developed for this purpose, is lacking. This, together with an estimation of the principal components of the body—muscles, bones, and fat—from the external dimensions of the body (J. Matiegka, *Am. J. Phys. Anthropol.* **4,** *223* (*1921*)) is a challenge to a functionally oriented physical anthropology. A simplified inspectional scale for the rating of man's physique was offered by T. K. Cureton ("Physical Fitness Appraisal and Guidance," *see esp. p. 120, C. V. Mosby, St. Louis* (*1947*)). He speaks—as is preferable from the point of view of nutriture appraisal—of muscular and skeletal development rather than of mesomorphy and ectomorphy. But he still clings to the term "endomorphic characteristics," used in constitutional anthropometry and which confuses rather than clarifies the issue of "fatness."

A NEW PANTOTHENIC ACID ANTAGONIST FOR ANIMAL EXPERIMENTATION

The discovery of antagonism between para-aminobenzoic acid and sulfanilamide has led to innumerable attempts to prepare "antimetabolites," *i.e.*, substances which deviate slightly in structure from an essential metabolite and which, for this reason, may be able to compete for an important place in the cell without being able to perform the function required, so that inhibition ensues.

Confirmation of the general applicability of this concept was obtained with "pantoyltaurine." This pantothenic acid analogue contained a sulfonic acid group in place of the carboxyl group of the vitamin (E. E. Snell, *J. Biol. Chem.* **139,** *975* (*1941*); R. Kuhn, T. Wieland, and E. F. Möller, *Ber. d. deutsch. chem. Gesellsch.* **74,** *1605* (*1941*)). Pantoyltaurine was found to inhibit growth of lactic acid bacteria by competing with pantothenic acid according to the law of mass action. The structure of pantothenic acid offers numerous possibilities for variations, and a host of other pantothenic acid analogues have been prepared. Many of these are true competitive antagonists, especially when tested against microorganisms, for example, lactic acid bacteria. None of them, however, had been reported to act as a pantothenic acid antimetabolite in animals. An early observation of pantothenic acid deficiency symptoms produced in mice by pantoyltaurine was not confirmed (D. W. Woolley and A. G. C. White, *Proc. Soc. Exp. Biol. Med.* **52,** *106* (*1943*); K. Unna, *Ibid.* **54,** *55* (*1943*)). The antagonist was probably nonspecifically and irreversibly toxic at the levels fed. The definition of an antimetabolite necessarily requires that its effects are prevented—and possibly also reversed—by treatment with the metabolite.

Promising results in animals have recently been described with omega-methylpantothenic acid. The substance contains a methyl group in place of a hydrogen atom on the terminal CH_2OH group of the pantothenic acid molecule. It has been synthesized from beta-alanine and alpha, gamma-dihydroxy-beta,beta-dimethylvaleric acid in the same fashion as the vitamin (A. H. Nease, Dissertation, *U. of Texas* (*1943*)). Earlier investigations had shown that omega-methylpantothenic acid exerted a strong growth-inhibiting effect on lactic acid bacteria and also on protozoa. It prevented growth of *Tetrahymena geleii* (*in vitro*) and had been tested unsuccessfully for antimalarial activity, using *Plasmodium lophurae* infection of chicks for the assay (L. A. Schinazi, W. Drell, G. H. Ball, and M. S. Dunn, *Proc. Soc. Exp. Biol. Med.* **75,** *229* (*1950*)).

In the latter paper, the authors reported incidentally that the substance was rela-

tively nontoxic for chicks, rats and mice, when given orally or intraperitoneally. A preliminary experiment showed that the effect of 0.5 per cent omega-methylpantothenic acid in a purified diet on the body weight of rats was completely prevented by inclusion of 0.01 per cent calcium-pantothenate. More detailed investigations were performed on mice of the Bagg strain (W. Drell and M. S. Dunn, *Arch. Biochem.* **33,** *110 (1951)*). The animals were raised on a purified pantothenic acid-free diet which was supplemented with the vitamin and the analogue in a variety of combinations. The appearance of the mice, their food consumption, body weight, and survival time depended upon the ratio between the amount of the inhibitor and the pantothenic acid in the diet. Pantothenic acid deficiency in Bagg mice produces ruffling and thinning of the hair, which within several weeks leads to progressive alopecia. Food intake is diminished and the animals fail to grow without, however, losing weight. Death occurs on the average after sixty days. Inclusion of as little as 60 mg. of omega-methylpantothenate in 100 g. of diet caused loss of weight and reduced the average survival time to thirty-four days. At 500 mg. per 100 g. of diet the survival time was only about sixteen days.

Pantothenic acid prevented these effects when given in amounts sufficient to exceed a certain ratio between antagonist and vitamin. The authors defined as "interference index" the proportion of inhibitor to vitamin at which survival time was equal to the controls on the vitamin-free diet. The interference index of approximately 100:1 was constant up to levels of 0.6 per cent omega-methylpantothenic acid. When more than 100 parts of the antagonist to 1 part of pantothenic acid were used, the development of the deficiency was accelerated, and when less than 100 parts of the antagonist were given, the animals survived, gained weight and did not show deficiency symptoms. The syndrome produced by the analogue was not only prevented but could also be cured when pantothenic acid was administered in amounts which brought the ratio of competitor to vitamin below the interference index.

The interference index of 100:1 for mice compares favorably with antagonist-to-vitamin ratios obtained with lactic acid bacteria. In many strains, omega-methylpantothenic acid prevents growth completely when used in the culture medium in ratios between 80:1 to 1000:1. With several other strains even higher concentrations of the inhibitor are necessary for the effect. There appears to be a common basis for the action of omega-methylpantothenic acid in bacteria and in animals.

G. D. Novelli and F. Lipmann have shown that certain pantothenic acid analogues inhibit the conversion of pantothenic acid to coenzyme A, the coenzyme of acetylation (*Fed. Proc.* **7,** *177 (1948)*). It is well known that in pantothenic acid-deficient animals acetylation of aromatic amines like sulfanilamide is decreased (T. R. Riggs and D. M. Hegsted, *J. Biol. Chem.* **172,** *539 (1948)*). Acetylation returned to normal after administration of the vitamin. M. E. Shils has investigated the effect of omega-methylpantothenic acid on the acetylation of sulfanilamide in pantothenic acid-deficient rats (*Proc. Soc. Biol. Exp. Biol. Med.* **75,** *352 (1950)*). It was found that the antagonist interfered with the restoration of normal acetylation, presumably by blocking the formation of coenzyme A.

Valuable results in nutrition and biochemistry can be obtained by the proper use of antimetabolites such as omega-methylpantothenic acid. The possibilities in this field seem to be incompletely realized thus far.

AMINO ACIDS AND LIPOTROPISM

In 1932 it was shown that rats on a diet moderately low in protein but rich in fat develop fatty livers in less than a month (C. H. Best, J. M. Hershey, and M. E. Huntsman, *J. Physiol.* **75,** *56* (*1932*)), but that choline added to the ration prevented the accumulation of lipid in the liver (Best and Huntsman, *Ibid.* **75,** *405* (*1932*)). It was later observed that as the amount of casein in a fatty liver-producing diet was increased, the glycerides in the liver decreased (A. W. Beeston, H. J. Channon, and H. Wilkinson, *Biochem. J.* **29,** *2659* (*1935*)), thus indicating that, in addition to choline and betaine, some constituent of protein was likewise capable of bringing about a mobilization of hepatic lipids. That this lipotropic factor is methionine was demonstrated by H. F. Tucker and H. C. Eckstein (*J. Biol. Chem.* **121,** *479* (*1937*)). Despite the fact that both choline and methionine possess methyl groups which have been shown to be labile and intimately associated with lipotropism, efforts have been made to demonstrate a similar action for other amino acids with somewhat equivocal results, particularly when the amino acid in question was added to a low protein diet.

In order to secure more convincing evidence regarding the lipotropic action of amino acids other than methionine, a recent study (H. C. Eckstein, *J. Biol. Chem.* **195,** *167* (*1952*)) was carried out using the "essential" amino acids minus methionine as the main source of nitrogen at a level of 9.53 per cent in the diet. Together with a mixture of pure vitamins, the remainder of the diet consisted of corn oil, cod liver oil, salts and carbohydrate. Control diets contained the foregoing basal ration with either 35 per cent or 5 per cent of casein. As noted earlier, fatty livers were produced in the control animals when the 5 per cent casein ration was used, whereas normal values for liver lipids were observed on the 35 per cent casein ration. When the amino acid mixture was used with 0.2 per cent methionine, fatty livers were produced, whereas if the methionine content was raised to 1.2 per cent normal values for liver fat were secured. The data strongly suggest that of the "essential" amino acids, methionine alone exerts a hepatic lipotropic action.

A second experiment was carried out in which the basal concentrations of the essential amino acids plus cysteine in the diet corresponded to the amounts available in the 5 per cent casein ration. (With the exception of DL-methionine and DL-phenylalanine, the DL-amino acids were included in twice the amount.) Cysteine was included because it is present in small amounts in a 5 per cent casein diet as commonly used in such studies, and because it is known to augment liver lipids when vitamin B-complex concentrates rather than pure compounds are fed. With no additional essential amino acid added to the above mixture (equivalent to the "essential" amino acids in a 5 per cent casein diet), the livers contained fat in augmented amounts similar to that in animals given the 5 per cent casein. Then each "essential" amino acid, in turn, was added separately in an amount corresponding to that in the 35 per cent casein basal diet; methionine stood out as the one amino acid showing pronounced hepatic lipotropic activity. There is a suggestion from the data that histidine and tryptophan exert some degree of lipotropic action, but in both cases the hepatic lipids are as high as with the 5 per cent casein control diet. Only methionine reduced the liver fat to the level observed when the 35 per cent casein control ration was fed. Employing such technics it appears, therefore, that among the essential amino acids only methionine exerts a lipotropic action in the liver.

VITAMIN NEEDS DURING REPRODUCTION

The need of the pregnant rat for thiamine and riboflavin was recently investigated by M. Barrett and G. Everson (*J. Nutrition* **45,** *493 (1951)*). These studies fill a definite need in that the quantitative requirements for satisfactory reproduction have not been examined very critically in the past.

These studies were conducted on healthy stock animals with satisfactory reproductive histories. The progressive transfer of vitamins from maternal tissues to the fetuses was studied in the second pregnancies of the stock animals. Assays for the vitamins in the maternal tissues and the uterus and its contents were made on the sixth, tenth, eleventh, and twelfth days of pregnancy. Similar assays were made at one-day intervals on the fetuses, placenta and maternal tissues from the thirteenth day of the gestation period until parturition. Only those females that produced 9 or more young per litter were used, and data on 3 animals were obtained at each of the time intervals indicated above.

Maximum growth of the fetus was observed to occur during the final four days of pregnancy, when the average weight of the fetuses increased from 13 g. to 55 g. per litter. The concentration of thiamine in the carcasses of the pregnant females remained constant between the sixth day of gestation and term (range, 1.9 to 2.2 micrograms per gram). Increased concentrations of thiamine were noted in maternal liver tissue in the late stages of pregnancy when the rapid fetal growth occurred. Values of 10 to 12 micrograms per gram of liver in early pregnancy increased to 14 micrograms in the last five days of pregnancy. The total amount of thiamine in the liver likewise increased during this period from about 100 micrograms to about 135 micrograms.

The concentration of thiamine present in early placental tissue ranged from 2.3 to 3.5 micrograms per gram. Since the amount of early placental tissue is small, it was concluded that the development of this structure did not materially influence the thiamine needs of the mother. The total thiamine present in the fetuses, per litter, on the fifteenth day of pregnancy was only 5.3 micrograms. This increased to 165 micrograms at full term and emphasizes the marked demands for thiamine near term.

The concentration of riboflavin in the maternal carcass remained unchanged during pregnancy and ranged between 2.4 and 2.8 micrograms per gram during the gestation period. Likewise, the liver concentration of riboflavin remained unchanged during gestation (range, 23 to 28 micrograms per gram). The riboflavin content of the fetuses increased from 3 micrograms per litter on the fifteenth day of pregnancy to 136 micrograms at parturition. These results again suggest that there is probably little need for riboflavin in early pregnancy beyond that required by the nonpregnant adult animal. The tremendously rapid increases in fetal riboflavin in the late stages of pregnancy emphasize the major increase in the vitamin needs of the mother during this period.

Food consumption records indicated that the pregnant females usually ingested between 150 and 180 micrograms of thiamine and between 100 and 120 micrograms of riboflavin daily.

As the stage of pregnancy advanced there was a progressive rise in food consumption and consequently a rise in thiamine and riboflavin intake. Urinary excretion of these vitamins, however, did not change greatly. This indicated that the increased vitamin intake in late pregnancy was probably utilized to meet fetal demands. From the data the authors calculated that by the twentieth day of gestation the thiamine needs were about 70 micrograms higher per day than the needs of the nonpregnant

female. Similar calculations indicated that the riboflavin requirement on the twentieth day of gestation was at least 28 micrograms above that of the nonpregnant adult female.

If the daily requirements for thiamine and riboflavin for adult rats that have been suggested are taken into consideration, it becomes apparent from this recent study that the demands of late pregnancy are rather considerable. The daily thiamine requirement during late pregnancy can be calculated to be about 103 micrograms as contrasted with the suggested level of 33 micrograms daily per adult nonpregnant rat (R. A. Brown and M. Sturtevant, *Vitamins and Hormones* **7,** *171* (*1949*)). The daily riboflavin requirement during late pregnancy can be calculated to be about 58 micrograms as contrasted with the suggested level of 30 micrograms daily per adult nonpregnant rat (*loc. cit.*).

In this connection it is of interest that the National Research Council recommended dietary allowance for riboflavin in the latter half of pregnancy (3.0 mg. daily) in humans is two-fold the recommendation for nonpregnant women, 1.5 mg. daily. The rat data would appear to conform with such a recommendation. On the other hand, the rat data suggest a much greater fetal and placental demand for thiamine near the end of pregnancy (three times the adult nonpregnant requirement) than is recommended for pregnant women. The recommended allowance in the latter half of pregnancy is 1.5 mg. daily, compared with an allowance of 1.2 mg. for moderately active nonpregnant women.

More quantitative data of the type reported by these workers are definitely needed to strengthen our knowledge of the nutritional demands during reproduction.

CARBONATE IN THE SYNTHESIS OF MILK CONSTITUENTS

Until recently, carbon dioxide fixation, the incorporation of carbonate into organic compounds, was regarded as characteristic of the chlorophyll-containing plants and of autotrophic microorganisms. In 1940, however, it was shown (S. Ruben and M. D. Kamen, *Proc. Nat. Acad. Sci.* **26,** *418* (*1940*)) that animal tissues were also capable of carbon dioxide fixation. The next year, A. K. Solomon and his collaborators (*J. Biol. Chem.* **140,** *171* (*1941*)) showed that carbonate could be utilized by the intact rat for the synthesis of glycogen. A recent report (M. Kleiber, A. H. Smith, and A. L. Black, *Ibid.* **195,** *707* (*1952*)) now indicates that carbonate can be a precursor of the three major components of cow's milk secreted under normal circumstances.

Two lactating Jersey cows were used in these experiments. Each was fed a ration of alfalfa hay and grain, and gave about 10 quarts of milk daily during the time of the study. The cows were milked out, then labeled carbonate (in the form of sodium bicarbonate containing C^{14}) was injected into the left jugular vein. The first milk samples were taken three or six hours after the injection and at twelve-hour intervals henceforth. The milk was kept at 4°C. for a day. The various constituents were then prepared as follows: The skim milk was siphoned off from beneath the hardened cream layer, from which the fat was extracted by the classic methods. Casein was precipitated from the skim milk by addition of acetic acid; lactoglobulin was obtained by neutralization of the supernatant and moderate heating. Lactose was then burned in an oxygen bomb and the activity of the carbon dioxide thus produced was measured for each collection of milk.

By addition of the results obtained for each fraction, Kleiber and his collaborators found that an appreciable proportion of the

carbon in lactose, casein and fat orginated from carbonate: it amounted to 10 per cent for the lactose, 4 per cent for the casein and 2 per cent for the fat. By determining the specific activity of the various constituents for each of the milk collections, it was also possible to show that the period of most rapid carbon dioxide incorporation varied according to the constituent. The specific activity of collected lactose, casein and albumin was found to reach its maximum during the first three hours after injection of the tracer. By contrast, milk fat showed maximum activity between three to nine and one-half hours after the injection. During the first thirty-three hours of the experiment it had the lowest activity of any of the milk constituents. However, in the last sample, corresponding to a collection taken almost two days after the tracer injection, fat had the highest specific activity and lactose the lowest. Differences in rates of synthesis of the various constituents are thus manifest. Furthermore, certain characteristics of the curves expressing the specific activity of the milk constituents as a function of time, lactose in particular, suggest to the authors the possibility that the production of these substances is the resultant of several coexistent types of biologic synthesis, each pathway involving different delays.

The authors indicate that study of carbonate incorporation into single amino acids of casein is in progress and that the results will be published in the near future. Further reports on this work, the importance of which is obvious from both a theoretic and a practical standpoint, will be awaited with interest.

NOTES

Letter to the Editor

Dear Sir:

I was gratified to find in the February issue of *Nutrition Reviews* (**10,** *42 (1952)*) a review of my paper entitled "Nutrient Requirements as Related to Body Size and Body Function" (*Scientia* **85,** *165 (1950)*), since a main purpose of the paper was to stimulate interest and discussion in a phase of nutrition that seems to be sadly neglected. However, more through my fault than that of the reviewer, the table entitled "The Nutrient Requirements of Adult Animals" has been misinterpreted. I am writing this letter to supply the necessary clarification.

The purpose of the table was to summarize the factored requirements, as they were discussed in the text of the article, for adult animals under nonworking and nonstress conditions, and the title should have been modified accordingly. Your reviewer naturally takes exception to the use of body surface as a reference for "unspecified muscular activity." The muscular activity referred to is the activity incidental to the nonworking adult animal; in the case of man, to a sedentary life. Rightly or wrongly, this increment in energy expenditure over and above the basal expenditure, to give a "maintenance" requirement of net energy, is commonly expressed as a percentage of the basal expenditure. I have chosen for this net energy item a value of 50 per cent of the basal metabolism, based upon a good deal of direct and indirect evidence that I have garnered from the literature. It is admittedly subject to variation, probably within a range of 25 to 100 per cent.

Again, largely on the basis of the review of E. F. Adolph (*Physiol. Rev.* **13,** *336 (1933)*), I have included in my table a water requirement of 1 ml. per calorie of heat produced. Your reviewer admits that "such an allowance is valid under comfortable environmental conditions." He then points out that, for the human being, much more water is required in a hot environment. It seems to be a good value for all adult warm-blooded animals when not working and when in a comfortable environment. Obviously, it will be too low an estimate for working animals, or animals exposed to high environmental temperatures, and probably too high for animals in a cold environment.

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New Scientific Journal

The appearance of a new research journal is not always received with unqualified enthusiasm by the present day scientific reader whose desk is already piled high with publications demanding attention. There is, however, a real need for an additional medium for the presentation of observations in the rapidly expanding field of experimental and clinical metabolism. *Metabolism* is a new bimonthly journal, the first issue of which appeared in January 1952, devoted to this area. It is published by Grune and Stratton, 381 Fourth Avenue, New York, and Dr. Samuel Soskin of Michael Reese Hospital, Chicago, is the editor. Many of its papers will deserve careful study by those interested in the field of nutrition.

Gordon Research Conference

On August 11–15 the Conference on Vitamins and Metabolism will be held at Colby Junior College, New London, New Hampshire. Requests for attendance or for any additional information should be addressed to W. George Parks, Director, Department of Chemistry, University of Rhode Island, Kingston, Rhode Island. After June 15, 1952, mail should be addressed to Colby Junior College, New London, New Hampshire.

Loss of Lysine During Bread Baking

The classic studies of T. B. Osborne and L. B. Mendel (*J. Biol. Chem.* **17,** *325 (1914)*; **37,** *557 (1919)*) revealed that the protein of wheat is deficient in the essential amino acid, lysine. Since wheat products, mainly in the form of bread, are very widely consumed by humans, efforts have been made to raise the lysine content. The practice of American bakers of adding 3 to 6 per cent nonfat milk solids to bread formulas raises the lysine content of the bread, but this amino acid is still limiting. Larger quantities of milk solids or of dried yeast in bread formulas as a means of increasing the lysine content have generally not been acceptable because the taste and texture of the bread are adversely affected.

Lysine, either the naturally occurring L-form, or the synthetic DL-form, has become available in sufficient quantities recently to justify studies concerning its stability when added to bread formulas. H. R. Rosenberg and E. L. Rohdenburg (*J. Nutrition* **45,** *593 (1951)*) have recently reported on the heat destruction of lysine in bread during baking.

A prepared commercial flour mix and commercial basic bread formulas were used as the starting materials in the first series of studies to determine the average loss of lysine present in flour and milk proteins during bread baking. It was found that lysine destruction occurred only on the surface of the loaf and was presumably associated with the browning of the crust. Total losses ranged from 2.4 per cent to 15.9 per cent with an average loss of 11.0 per cent.

The regular and the lysine-fortified loaves were baked side by side. In the bread to which DL-lysine hydrochloride had been added at a level of 0.5 per cent (equivalent to 0.2 per cent L-lysine), the loss of the added lysine during baking averaged 15.4 per cent (range 9.5 to 23.8 per cent). L-lysine hydrochloride added at a level of 0.25 per cent (equivalent to 0.2 per cent L-lysine) resulted in baking losses of 32 per cent (range 25 to 36.8 per cent). Since the microbiologic method used in lysine analysis measured only the L-form, the greater loss when only the active L-form was added would imply that regardless of the compound used for fortification an equal number of molecules of lysine were destroyed in baking. Toasting a slice of bread reduced the lysine content by 5 to 10 per cent, and a similar loss occurred when bread became stale and dry. The lysine fortification levels used raised the lysine content of the bread from approximately 0.40 per cent to approximately 0.60 per cent (dry basis) and this addition imparted a slight change in taste variously described as "more salty" or "more tasty."

These results appear to warrant the conclusion that breads can be fortified with lysine, but that the loss of added lysine is somewhat greater than the loss of the lysine naturally present in flours or bread mixes.

Japanese Institute of Nutrition

The National Institute of Nutrition, Toyamacho, Tokyo, Japan, has recently published the annual report of its activities for the year 1949–1950. The report briefly summarizes some 28 projects in nutrition which it has under way. These deal with a great variety of subjects, ranging from a nutrition survey of policemen to experimental studies on specific dynamic action. Dr. Arimoto, the Director, points out that the Institute was established in 1920 and started with a staff of 24 members and a budget of about 100,000 yen a year. The Institute was nearly completely demolished during 1945. However, in May of 1947 it moved to a new location and now has 63 staff members and a yearly budget of 10,500,000 yen.